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12/17/2018 – *Open Access*

The Mapping of *Drosophila melanogaster* mutant *A.4.4*

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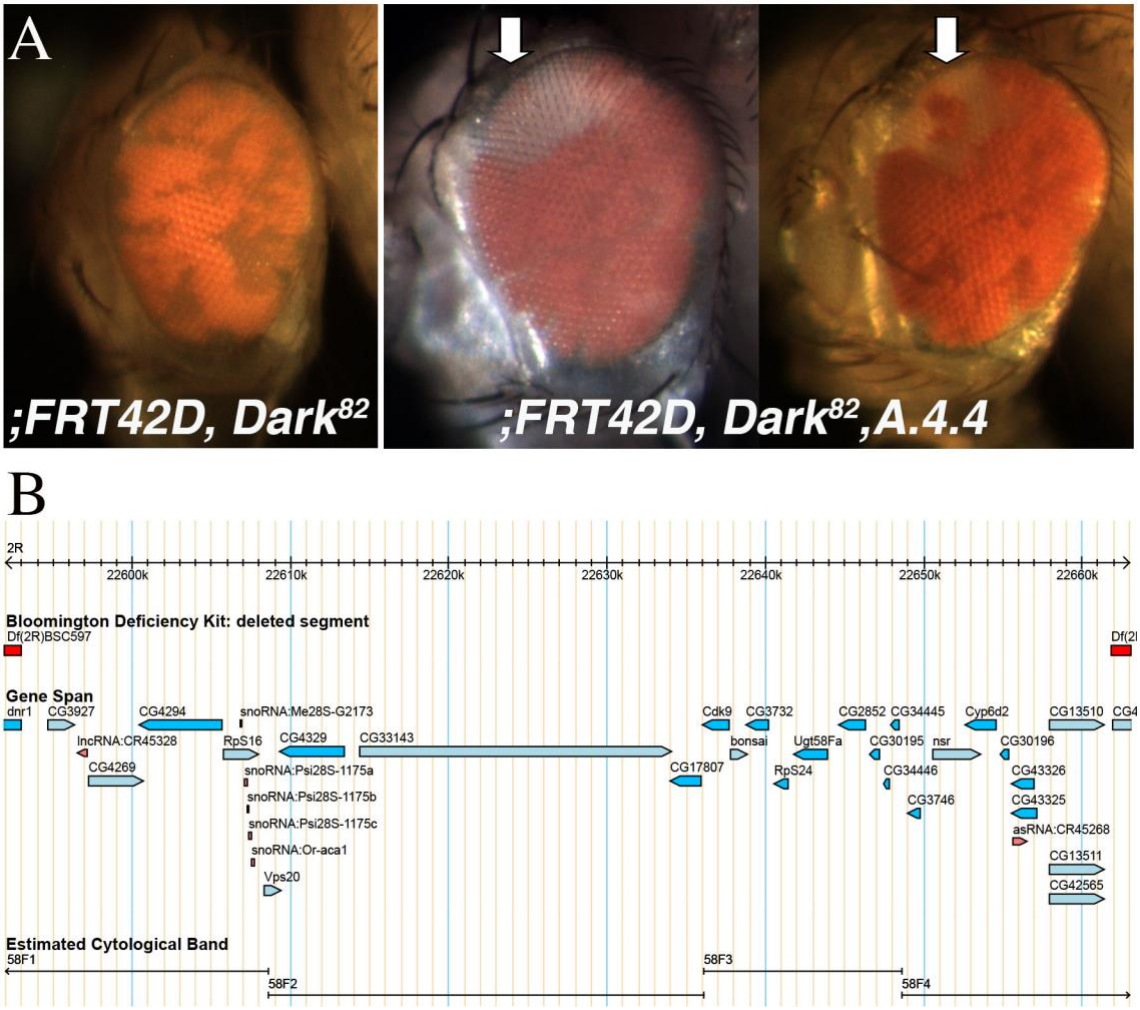

Figure 1 *A. Mosaic eye of control (FRT42D Dark^{82}) and mutant A.4.4 (FRT42D Dark^{82} A.4.4) flies, mutant tissue is pigmented (mw^{+}). Two representative mosaic eyes are shown for A.4.4. Arrow denotes consistent patch of wild type tissue observed on dorsal tip of mosaic eye. B. Genomic region on Chromosome 2R in which mutant A.4.4 fails to complement, 2R:22,592,996..22,661,827. Image adapted from Flybase.org (Gramates et al., 2017).*

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Description

A novel *Drosophila melanogaster* mutant *A.4.4* was isolated from a conditional Flp/FRT mosaic eye screen in the context of blocked apoptosis (Kagey *et al.*, 2012). The *;FRT42D, Dark*^{82} chromosome was used as a starting point for the EMS mutagenesis screen to screen for mutations that conferred a growth advantage in the environment of blocked apoptosis via the homozygous *Dark*^{82} allele (Akdemir et al., 2006). Mutants were screened for over-representation of mutant tissue (pigmented) as compared to the *Dark*^{82} mosaic control (Figure 1A). The mutant mosaic phenotype generated by the cross *FRT42D Dark*^{82} *A.4.4* X Ey>Flp; FRT42D resulted in mosaic eyes with a slight increase in the red:white ratio (approximately 70:30) as compared to *FRT42D Dark*^{82} control eyes (approximately 60:40). Ratios were estimated from observation of multiple mosaic eyes for each genotype. In addition to the increase in mutant tissue, the mosaic *A.4.4* eye was observed with a consistent clone/patch of wild type (unpigmented) tissue at the dorsal peak of the eye (**Figure 1A,** arrow denotes observed region lacking mutant tissue). Whether this mutant phenotype is dependent upon this block in apoptosis is unknown at this time, however other mutant phenotypes in this screen have demonstrated a dependence upon a block in cell death (Kagey *et al.*, 2012).

The genomic location of the homozygous lethal *A.4.4* was mapped by deficiency mapping and complementation tests to identify the region on 2R that failed to complement. The location of the mutation was mapped by three independent groups of researchers that are part of the Fly-CURE consortium utilizing complementation mapping and the Bloomington Stock Center 2R Deficiency Kit (Cook *et. al.*, 2012). We find that mutant *A.4.4* failed to complement the deficiency *Df(2R)X58-12/SM5.* Mutant *A.4.4* complemented the overlapping deficiencies *Df(2R)BSC597/SM6a* and *Df(2R)BSC787/SM6a*. Together these data create a failure to complement region of 2R:22,592,996..22,661,827 (**Figure 1B**). Additional complementation tests were set up with individual alleles of candidate genes found within this region and available at the BDSC and tested for lethality (**Table 1**). All of these crosses to individual alleles complemented *A.4.4* suggesting that the mutation resides in one of the other genes within this genomic region. The initial complementation experiments were conducted in triplicate at three independent institutions, while the individual allele complementation tests were conducted once. In summary mutant *A.4.4* mapped to 2R:22,592,996..22,661,827 via deficiency mapping where there are 29 protein coding genes. After additional complementation tests ruled out eight additional genes, *A.4.4* likely resides in one of the remaining 21 genes in the region that we were unable to map via complementation.

Table 1: Complementation between mutant *A.4.4* and individual candidate alleles

Stock number BDSC	Gene affected	Genotype	Mating with A.4.4
12060	*Vps20*	*P{PZ}Vps20*^{rG270}*, l(2)rG270rG270/CyO*	Complement
16199	*CG4294*	*y*^{1} *w*^{1118}*; PBac{5HPw*$^{+}$*}CG4294*^{B316}*/CyO*	Complement
17065	*CG3927*	*w*^{1118}*; P{w*$^{+mC}$*=EP}EP2515/CyO*	Complement
17739	*Ugt58Fa*	*w*^{1118}*; PBac{PB}Ugt58Fa*^{c05973}*/CyO*	Complement
23049	*CG33143*	*y*^{1} *w*^{67c23}*; Mi{ET1}CG33143*^{MB01293}*/CyO*	Complement
29511	*RpS24*	*w*; P{FRT(whs)}G13 P{lacW}RpS24*^{SH2053}*/CyO*	Complement
63874	*RpS16*	*w*^{1118}*; PBac{IT.GAL4}RpS16*$^{0887-G4}$*/CyO*	Complement
67706	*Vps20*	*w*; Vps20*^{I3}*/CyO*	Complement

Reagents

FRT42D Dark^{82}*/CyO* (Akdemir et al., 2006)
FRT42D Dark^{82} *A.4.4/CyO*
Ey>Flp; FRT42D (BDSC 5616)
Bloomington Drosophila Stock Center 2R Deficiency Kit (Cook *et al.*, 2012)
Individual alleles used for complementation tests (see Table 1 for BDSC numbers)

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Funding source None

Reviewed by Andy Zelhof

Received 09/10/2018, **Accepted** 12/15/2018. **Published Online** 12/17/2018.

Citation: Bieser, K. L., Stamm, J., Aldo, A. A., Bhaskara, S., Claiborne, M., Coronel Gómez, J. N., … Kagey, J. D. The Mapping of Drosophila melanogaster mutant A.4.4. microPublication Biology. https://doi.org/10.17912/MICROPUB.BIOLOGY.000069